LBNL Sustainable Systems SFA: Exploratory Project

ENHANCED IMMOBILIZATION OF METALS AND RADIONUCLIDES IN THE VADOSE ZONE

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ABSTRACT

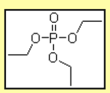
Significant quantities of metals and radionuclides are contained in unsaturated rocks at several DOE sites in the western US. In many cases, this contamination has migrated to groundwater, sometimes decades after being released into the subsurface (Christensen et al., 2005). Immobilizing these contaminants in the vadose zone could greatly reduce the threat they pose to groundwater and decrease the costs of closing these sites. This exploratory project is focused on stimulation of biologically enhanced phosphate mineralization under unsaturated conditions through injection of gas-phase organophosphate compounds. Phosphate minerals will incorporate contaminants such as U and ^{90}Sr into their mineral structure. To induce precipitation of phosphate minerals in unsaturated sediments, we are testing the use of gas-phase compounds for delivery of the necessary chemicals to the vadose zone. Our initial studies are focused on tri-ethyl phosphate (TEP), which has a moderate vapor pressure, is miscible with water, and has low toxicity. To accelerate release of phosphate from TEP, we are testing methods of stimulating microbial degradation of TEP.

Our current research is concentrated on determining the chemical and biological properties of TEP and phosphate in materials from the Hanford Site and the INL (see summaries of the results of this work to the right). Chemical studies have included a series of experiments to measure the rates of aqueous phosphate adsorption on Hanford sediments and kinetic experiments designed to determine the concentrations of phosphate in solution necessary to induce mineral precipitation. In addition, we have been conducting two sets of long-term biodegradation experiments with TEP. To identify microorganisms capable of metabolizing TEP in unsaturated sediments, enrichment cultures derived from sediments from the INL vadose zone have been growing in simulated groundwater with 5 mmol TEP for one year. A second set of biodegradation experiments are being conducted with slurries of Hanford sediment also containing 5 mmol aqueous TEP.

BACKGROUND

Chemical Properties of Triethylphosphate (TEP)[1]

Chemical Formula: $(C_2H_5)_3PO_4$
Molecular weight: 182.15 g/mol
Density: 1060 kg/m^3
Vapor Pressure: 385 ppmv at 20 °C
Photodegradation: $T_{1/2}$ in air = 8.8 hrs
Stability in water[2]: $T_{1/2}$ = 5.5 years



[1] Also known as Phoster® (DOE Assigned Trademark & US patent 5,480,549 Looney et al., 1996 for Bioremediation).
[2] For a half life for hydrolysis in water of 5.5 years, ~0.6 mmol of phosphate should have been generated in the kiiled controls with TEP for the INL degradation experiments. However, none was observed, suggesting that the actual half life is much longer than 5.5 years.

Phosphate has been added to groundwater systems contaminated hydrocarbons and/or chlorinated solvents to stimulate stimulate microbial activity. Inorganic phosphate (K_3PO_4) is sometimes used, but can lead to such high levels of activity near the injection point that the phosphate does not get distributed throughout the system and sometimes clogs up the injection well with biomass. To overcome these issues, organophosphate compounds such glycerol-3-phosphate, tributyl phosphate (TBP), and TEP have been used for this purpose. These compounds are soluble in water and will diffuse out from the injection well. The phosphate in these compounds is also less bio-available, allowing them to travel further away from the well. Phosphate compounds have also been added to groundwater systems contaminated with metals and radionuclides in order to stimulate precipitation of phosphate minerals that will remove the contaminants from the groundwater.

Treating contamination in the vadose zone presents additional challenges. It is generally not practical to emplace liquid or solid additives into unsaturated rocks, especially when the contamination is not near the land surface. Addition of TEP gas has been successfully used to stimulate microbial activity in hydrocarbon-contaminated unsaturated systems that are phosphorus limited. However, they do not prove that the organisms were producing enough phosphate to cause precipitation of phosphate minerals or, if they were, to what extent did the phosphate minerals incorporate trace metals, such as strontium or uranium into their crystal structure. An important goal of the research proposed below is to demonstrate that this is happening.

HYPOTHESES

The primary hypotheses that we are testing with this research are the following:

- Given optimal conditions, natural subsurface microbial communities can accelerate degradation of TEP in unsaturated sediments.
- Sufficient phosphate can be delivered to vadose zone pore waters through injection of TEP-saturated air to achieve precipitation of phosphate minerals.
- The resulting phosphate minerals incorporate significant concentrations of the target contaminants (strontium-90, uranium).

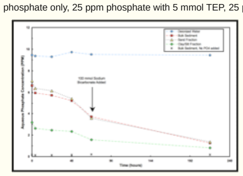
APPROACH

These hypotheses are being tested through a series of experiments examining the response of the microbial communities to addition of TEP and other nutrients such as ethanol and N_2O. The emphasis of experiments during this year is to identify microorganisms from target sites capable of metabolizing TEP and to quantify other geochemical factors that will affect delivery of amendments and phosphate mineral precipitation. The microbial communities will be monitored using Phylochip analyses and other molecular techniques. The fate of the amendments used during these experiments and the uptake of target contaminants into precipitates will be evaluated using isotopically-labeled tracers (e.g., ^{18}O-phosphate, ^{87}Sr) and synchrotron analyses of any materials formed during the experiments. Research during the second year will focus on quantifying biological and abiotic rates of degradation of TEP and other organophosphate compounds, production of inorganic phosphate, and precipitation of phosphate minerals in unsaturated column experiments. Concurrent with the laboratory experiments, a predictive reactive transport model will be developed to examine the potential rates of contaminant removal during TEP injection based on the results of the experiments.

RESULTS

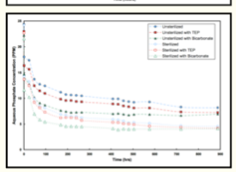
Phosphate Adsorption onto Hanford Sediments

Experiments are being conducted to determine the degree of adsorption of phosphate to Hanford sediments. Two sets of experiments have been done; one to test the effect of size fraction on adsorption to 2:1 mixes of de-ionized water with 10 ppm phosphate and Hanford sediments sterilized by gamma irradiation and a second set to test differences between sterilized and unsterilized sediment with different amendments (25 ppm phosphate only, 25 ppm phosphate with 5 mmol TEP, 25 ppm phosphate with 100 mmol Na-bicarbonate).



Size Fraction Experiments

- Sorption to the clay/silt fraction was more rapid, but with time, the degree of sorption to the sand fraction and the bulk sediment approached that of the finer fraction.
- Addition of 100 mmol Na-bicarbonate after 96 hours did not result in significant desorption of phosphate (although increased desorption of nitrate from the sediments was observed).



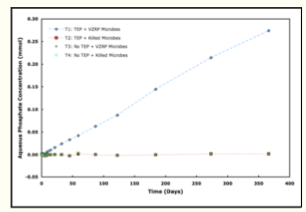
Amendment Experiments

- For all treatments, adsorption of phosphate to the sterilized sediment was significantly greater. Desorption of nitrate was higher for sterilized sediments.
- For the 3 treatments, adsorption was greatest with Na-bicarbonate added, second highest with TEP added and least with phosphate only.

RESULTS (CON'T)

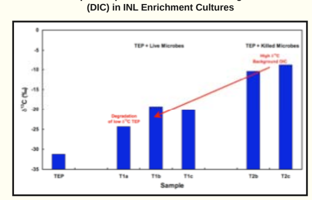
Degradation of TEP by INL Enrichment Culture

A series of long-term TEP degradation experiments have been run with an enrichment culture derived from the INL Vadose Zone Research Park (VZRP). Initial slurries were set up with VZRP sediments (collected 50-60 ft bgs) provided 10 mmol TEP as the sole source of carbon and phosphorus. After 3 transfers (with solids removed), the culture was transferred into INL synthetic groundwater amended with 5 mmol TEP for the experiments shown here. Each treatment was done in triplicate, in brown glass bottles with mininert caps, shaking at room temperature (22 ºC). Aerobic conditions were maintained by injecting 10 ml of room air into the head space of the bottles during every sample event.



- Phosphate concentrations in live cultures steadily increased to 0.27 mmol after one year (versus no detectable phosphate generation in killed cultures).
- From an initial cell density ~1.5 x 10^6 cells/ml, the average cell density in live cultures with TEP increased to 4.0 x 10^7 cells/ml versus an average cell density 2.9 x 10^6 cells/ml in the live cultures without TEP.

Carbon Isotope Compositions of Dissolved Inorganic Carbon (DIC) in INL Enrichment Cultures



- The carbon isotope ration of TEP (-31‰) is much lower than background inorganic carbon in the culture experiments, providing a natural tracer for microbial respiration of TEP.
- The $\delta^{13}C$ of DIC from the live cultures with TEP was ~12‰ lower than the $\delta^{13}C$ of DIC in the killed cultures confirming that significant degradation of TEP occurred in the live culture.

Hanford Slurry Experiments

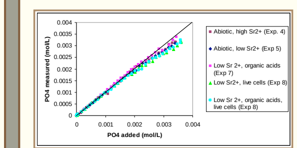
Experimental Setup – Slurry cultures were grown with 50 g of subsurface sediments from Hanford in 100 ml sterile deionized water with and without 5 mmol TEP. Gamma irradiated sediments were used to set up the sterilized controls. The slurries have been incubating for 9 months.

- Plate counts rapidly increased to greater than 10^6 colonies in the cultures with and without TEP but began dropping off after the first 6 weeks. Numbers of colonies in the culture with TEP have stayed higher than the cultures without TEP, possibly indicating some growth on the TEP.
- No significant accumulation of phosphate has been observed during these experiments (not unexpected given the results of the adsorption experiments), but there have been measurable decreases in TEP concentrations in the slurries. Although the total cell concentrations in the slurries have decreased, *Arthrobacter sp.* have become the dominant cultivable microorganisms present.
- A culture independent method using a high-density microarray (Phylochip) will be used to characterize the microbial communities in both the groundwater and slurry experiments.

RESULTS (CON'T)

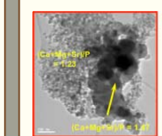
Phosphate Precipitation Experiments

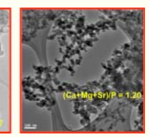
Inorganic phosphate was added to simulated INL groundwater (see composition below) to test the degree of oversaturation necessary to begin precipitation of phosphate minerals, the mineral phases produced and the degree of uptake of strontium. Despite a predicted solubility of 5x10^{-6} mmol for hydroxyapatite, precipitation was not observed until aqueous concentrations of ~2 mmol phosphate were reached. When these precipitation experiments were repeated with live bacterial cells, the apparent degree of over-saturation required to induce precipitation of phosphate minerals decreased slightly, possibly due to the availability of cell surfaces as heterogeneous nucleation sites.



Simulated INL Groundwater

	mmol/L
Na^+	1.97
Mg^{+2}	0.708
Ca^{+2}	1.3 - 1.6
K^+	0.124
HCO_3^-	0.649
SO_4^{-2}	0.242
Cl-	5.17
NO_3^-	0.414
Sr^{+2}	0.003 - 0.3





Mineral Precipitation

Solids precipitated in Experiment 4 with high Sr concentrations appear to be consistent with brushite whereas those precipitated in Experiment 5 with lower Sr concentrations appear to be more consistent with hydroxyapatite. That Sr may act to inhibit HAP nucleation is reported in the literature (Christoffersen et al, 1997).

SUMMARY

The results of these experiments indicate that TEP can be biodegraded by microorganisms found in unsaturated environments, but the rates may be very slow and the degree of over-saturation of phosphate necessary for precipitation of phosphate minerals may be large. However, given the slow rates of contaminant migration within the vadose zone, this may still represent a viable mechanism for *in situ* immobilization of contaminants. Future research will include identification of key organisms capable of degrading TEP and investigation of approaches to accelerate TEP degradation, for example by the provision of supplemental nutrients.

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